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Envelope–Receptor Interactions in Nipah Virus Pathobiology

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ABSTRACT: Nipah (NiV) and Hendra (HeV) viruses are members of the newly defined *Henipavirus* genus of the *Paramyxoviridae.* Nipah virus (NiV) is an emergent paramyxovirus that causes fatal encephalitis in up to 70% of infected patients, and there is increasing evidence of human-to-human transmission. NiV is designated a priority pathogen in the NIAID Biodefense Research Agenda, and could be a devastating agent of *agro*bioterrorism if used against the pig farming industry. Endothelial syncytium is a pathognomonic feature of NiV infections, and is mediated by the fusion (F) and attachment (G) envelope glycoproteins. This review summarizes what is known about the pathophysiology of NiV infections, and documents the identification of the NiV receptor. EphrinB2, the NiV and HeV receptor, is expressed on endothelial cells and neurons, consistent with the known cellular tropism for NiV. We discuss how the identification of the henipahvirus receptor sheds light on the pathobiology of NiV infection, and how it will spur the rational development of effective therapeutics. In addition, ephrinB3, a related protein, can serve as an alternative receptor, and we suggest that differential usage of ephrinB2 versus B3 may explain the variant pathogenic profiles observed between NiV and HeV. Thus, identifying the NiV receptors opens the door for a more comprehensive analysis of the envelope–receptor interactions in NiV pathobiology. Finally, we also describe how galectin-1 (an innate immune defense lectin) can interact with specific N-glycans on the Nipah envelope fusion protein, underscoring the potential role that innate immune defense mechanisms may play against emerging pathogens.

KEYWORDS: paramyxovirus; ephrinb2; receptor; envelope; tropism

NIPAH VIRUS: A LETHAL EMERGING VIRAL PATHOGEN

In 1999, Nipah Virus (NiV) was discovered to be the causal agent of an outbreak of respiratory illness in pigs and encephalitis in humans.[1–3] In 2001–2005, NiV outbreaks occurred in Bangladesh,[4–7] where pigs were not found to

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be infected, suggesting that the virus may have jumped directly from its natural host, the fruit bat or flying fox (genus *Pteropus*), to humans.[8–10] NiV infection typically leads to death from fatal encephalitis in humans, with a 40% to 70% mortality rate in the Malaysian and Bangladesh outbreaks, respectively.[11,12] The latter mortality rate is similar to that seen with viral agents, such as Ebola virus, and far exceeds the mortality associated with any of the recent emerging viral pathogens in South East Asia (e.g., SARS coronavirus and H5N1 influenza). While most clinically apparent cases in Malaysia were found in patients with direct exposure to infected pigs,[13] seroconversions and subclinical brain lesions were documented in exposed health care workers, demonstrating that human-to-human transmission of the virus is possible.[14] Indeed, there is stronger evidence of human-to-human transmission in the recent Bangladesh outbreaks.[11,15] NiV is a BSL-4 pathogen and is designated a Category C priority pathogen in the NIAID Biodefense Research Agenda.

BIOTERRORISM CONCERNS

NiV has been cited as a potential agent of bioterrorism due to several properties .[16] Its extreme pathogenicity (∼40–70% mortality) exceeds that of smallpox (∼30% mortality) and approaches that for Ebola (∼40–90%). In addition to causing acute disease, 3–7% of infected patients exhibit a late onset or relapsed encephalitis months to years after the initial infection,[14,17,18] thereby increasing the risk of community exposure. This is particularly worrisome given that there is increasing evidence of human-to-human transmissions in the most recent outbreaks.[11] NiV can be grown to high titers *in vitro* ($>10^8$ IU/mL without concentration) ,[14] and thus, it has been suggested it can be weaponized as an aerosol.[16] In addition, NiV may be a more devastating agent of *economic* or *agrobioterrorism* when used against the pig farming industry. During the NiV outbreak in Malaysia, which affected 265 individuals, more than 1 million pigs were culled, resulting in economic losses that totaled far more than their export value of US$100 million.[16] In the United States, the total annual production value of hogs and pigs in 2002 exceeded $8 *billion,* with farms in just three states (Iowa, Minnesota, and North Carolina) accounting for 50% of the value .[19] If Nipah-like agents were to be intentionally released in any one of these three states, the economic devastation from the loss of production alone could easily be more than $1 billion. This is not an unrealistic scenario; while the mortality rate in pigs (∼5%) is lower than in humans, the morbidity and transmission rate approaches 100%.[2,20] The observation that seroconversions and MRI-detectable brain lesions have been documented in exposed health care workers,[21] and in military personnel involved in the Malaysian culling operation, and that 30% of seroconverters went on to develop encephalitic sequelae,[22] further underscores the need to understand the pathogenesis of this disease.

NIPAH VIRUS BIOLOGY

As a member of the *Paramyxoviridae* family, the NiV contains a negative strand, nonsegmented, RNA genome, with a gene arrangement similar to other family members.[23] However, phlyogenetic analysis of NiV multiple genes suggests a clustering with the Hendra virus (HeV),[24,25] another paramyxovirus discovered a few years prior in Australia that caused an outbreak of severe respiratory syndrome in thoroughbred horses.[1,26] Functional and immunologic data also support the grouping of NiV and HeV into a new genus, as evidenced by the heterotypic complementation between their fusion (F) and attachment (G) envelope glycoproteins,[8,27] and the cross-reactivity between their respective sera.[24]

The NiV (and HeV) virus exhibits an unusually broad host range including humans, pigs, dogs, cats, horses, guinea pigs, hamsters, and fruit bats (its presumptive natural host).[2,28–30] Such broad host tropism is rare among extant paramyxoviruses. With the possible exception of fruit bats, disease mortality of all other hosts has been shown during natural or experimental infection.[30] However, despite evidence implicating pigs as the amplifying intermediate reservoir, the mortality in pigs ($< 5\%$) is far lower than that in humans (40–70%),[1,2] suggesting that zoonotic transmission to humans has increased the pathogenicity of the virus. Understanding the determinants of such broad tropism, but narrower pathogenicity, is critical for understanding the etiology of the disease. Since cellular tropism is determined in large part by the specificity of receptor–envelope interactions, identifying the NiV and HeV receptor is the first step toward this goal. Indeed, identification of the cognate receptor for NiV and HeV entry will likely speed the rational development of effective therapeutics against this deadly emerging pathogen.

As the requirement for studying this virus under BSL-4 conditions would understandably limit our ability to gain insights into the biology of this new genus of *Paramyxoviridae*, researchers have taken advantage of the fact that endothelial cell syncytia formation is a pathogenic hallmark of Nipah viral disease (see below), and is strictly mediated by the F and G envelope glycoproteins.[8,21,27] Thus, we and others have generated systems and reagents to study the determinants involved in viral envelope-mediated entry at less than BSL-4 conditions.[8,27,31–33] Notably, fusion assays and pseudotyping the NiV and HeV envelope glycoproteins on a replication incompetent vesicular stomatitis virus (VSV) reporter virus, allow studies of envelope-mediated membrane fusion and viral entry at less than BSL-4 conditions.

NIPAH VIRUS PATHOPHYSIOLOGY

As mentioned above, NiV emerged among agricultural workers in Malaysia in 1998. Among the 265 reported cases in the first outbreak, there were 105

patient deaths.[18] While most patients presented with a severe acute encephalitic syndrome, many also had significant pulmonary symptoms. Viral particles were obtained from cerebral spinal fluid (CSF) and characterized morphologically, serologically, and genetically, and the new virus was named after the Nipah River Village where the first isolates were obtained.[2,3]

The careful pathological investigations of 32 patients who died of NiV infection, provide a reasonably comprehensive description of NiV pathophysiology.[18] Several critical points will be summarized here. First, NiV kills infected patients very rapidly; the average time from fever onset to death was 9.5 days, and only 4/32 patients survived >14 days before death. Patients presented with fever (100%) and other neurologic symptoms (drowsiness, headache, disorientation). About 40% patients presented with respiratory symptoms.

Second, a major cellular target of the NiV appears to be endothelial cells that line blood vessels. At autopsy, microscopic evaluation revealed widespread vasculitis, endothelial cell destruction, and focal perivascular necrosis in small vessels in the lung (62%), heart (31%), kidney (24%), and central nervous system (CNS; 80%), with the most severe damage seen to vessels in the CNS. Importantly, syncytial or multinucleated giant endothelial cells were seen in blood vessels in many organs, frequently accompanied by vascular inflammation. This endothelial syncytia formation has been described as "perhaps the most unique histopathological finding," a feature not described in viral encephalides caused by other virus families. Importantly, in HeV-infected horses, increased numbers of endothelial syncytia have been associated with decreased survival.[34,35] Infection of endothelial cells and subsequent syncytia formation and endothelial damage has been proposed to trigger the thrombosis and necrosis seen in involved vessels. Thus, host tropism and fusogenicity of the viral glycoproteins [8,18,30] are likely to be significant determinants of viral pathogenicity.

Third, in addition to the endothelial and end organ damage, there was extensive lymphoid necrosis and immunologic detection of virus in lymphoid tissues, with lymphocyte apoptosis and detection of multinucleated giant cells in the lymphoid parenchyma and the subcapsular sinus. The authors have proposed that the exposure of the virus to cognate B cells probably occurs first in lymphoid tissues, given the observation that antibodies to the virus appeared in serum before appearing in the CSF. However, the target cells in lymphoid tissues have not been identified; the recent discovery of the NiV receptor(s) may shed light on this critical unanswered question.

Based on their findings, the authors suggested a temporal mode of events. Upon infection, primary replication of the virus occurs in lymphoid tissues (acute lymphoid necrosis), which leads to viremia and secondary replication in endothelial cells. Subsequent syncytia formation is the sentinel pathogenic event that leads to vasculitis, thrombosis, and necrosis and infection of parenchymal cells in various tissues. However, even though NiV antigen can be found in the microvasculature of many tissues, the highest antigen load

is found in the brain parenchyma, especially in neurons. Thus, neuronal and endothelial cell tropism is an established hallmark of NiV. The recent discovery of ephrinB2 as the receptor for NiV entry (see below) is remarkably consistent with the known tropism of NiV, as ephrinB2 is critically involved in neurogenesis and angiogenesis, and is highly expressed in neurons and endothelial cells. Ephrin genes are highly conserved, and have been found in all animal species examined.[36] Thus, the conservation of ephrinB2 may also explain the unusually broad tropism of NiV. EphrinBs bind to a large family of ephB receptor tyrosine kinases. It is the cognate bidirectional signaling that occurs during ephrinB–ephB interactions that direct the migration of endothelial cells and neuronal dendrites.[36–40] The interference with these interactions by NiV-G or HeV-G may also have great relevance for the pathogenicity of the virus.

NiV also has many unusual features, some of which undoubtedly contribute to its severe pathogenicity. For example, the NiV P, V, and W proteins, produced from the same P gene by variant RNA editing, have been demonstrated to exhibit anti-interferon functions by a variety of novel mechanisms.[41,42] These anti-interferon functions may dampen the initial innate immune response against this pathogen and contribute to the severe pathogenicity associated with this virus. However, whether the anti-interferon functions of NiV's P, V, and/or W proteins are qualitatively or quantitatively different from other paramyxoviral homologs remains to be seen.

DISCOVERY OF EPHRINB2 AS THE RECEPTOR FOR NIPAH AND HENDRA VIRUS ENTRY

Border and colleagues had previously demonstrated that NiV and HeV likely use the same cellular receptor, as fusion assays showed that NiV and HeV envelope glycoproteins exhibit the same tropism for a variety of cell lines.[8] These initial experiments suggested that the receptor was widely expressed, but not ubiquitous, as many human hematopoietic cell lines were not permissive for NiV and HeV envelope-mediated fusion. However, these initial experiments did identify several cell lines that were nonpermissive for viral fusion. In separate experiments, using a viral overlay technique to identify candidate proteins that may bind to NiV envelope glycoproteins, this group identified a protein candidate around 50 kDa that was bound specifically by viral particles in their viral overlay assay.[43] Then, using microarray analyses to compare a series of permissive and nonpermissive cell lines, Border and colleagues narrowed down a candidate list of putative receptors that fit several criteria (protein size, predicted membrane protein, highly expressed in permissive but not nonpermissive cell lines). Each of these candidate genes was then transfected into a nonpermissive cell line and tested for its ability to confer fusion permissivity by NiV envelope glycoproteins. Only one of the genes,

ephrinB2, was able to confer fusion permissivity. EphrinB2 was subsequently confirmed to be a bonafide receptor in their hands as it enabled live NiV entry into otherwise nonpermissive cell lines.[44]

Concurrently, the author's laboratory had also identified ephrinB2 as the bonafide NiV entry receptor by completely independent means.[31] We had first codon-optimized the ectodomain of the NiV attachment protein (G) and fused it to the constant Fc region of human IgG1. This was a necessary technique as paramyxoviral envelope genes are generally not expressed well from standard RNA Pol II-dependent promoters, at least not at levels required for substantive biochemical studies. Indeed, a large number of studies on paramyxoviral envelopes had relied on vaccinia-driven envelope expression. Thus, our studies on the properties of the Nipah envelope proteins can be performed under a less confounding experimental setting, as it has been noted that the use of vaccinia vectors in studies of paramyxovirus fusion necessitates the consideration of vaccinia structural proteins as a confounding factor,[45] although to our knowledge, reports of actual vaccinia-related confounding factors have not been reported. Our codon-optimized immunoadhesin (NiV-G-Fc) had cell –line-binding properties that recapitulated the published cell line tropism of NiV. Preparative immunoprecipitation followed by mass spectrometric analyses (LC-tandem MS/MS) leads to the identification of ephrinB2 as the protein that was specifically bound by the attachment protein of NiV (NiV-G). Several lines of evidence were used to show that ephrinB2 was the receptor for NiV entry: (1) soluble NiV-G binding to a variety of permissive cells was inhibited by soluble ephrinB2 and a variety of soluble ephB receptors, which are cognate endogenous receptors that bind ephrinB2; (2) transfection of ephrinB2 into a variety of nonpermissive cell lines made them permissive for NiV envelope-mediated fusion and entry; and (3) NiV envelope-mediated viral entry into endothelial cells and neurons, two primary cell types known to be infected by NiV *in vivo*, were inhibited by soluble ephrinB2.[31] Significantly, this latter point provided strong evidence that ephrinB2 is likely the *bonafide* cellular receptor used by NiV *in vivo*.

The ability to detect a specific interaction between the recombinant soluble NiV attachment envelope glycoprotein and a soluble recombinant version of its receptor suggests that this interaction can be exploited to screen chemical libraries in an ELISA-based sort of assay to identify leads for small molecule inhibitors of NiV or HeV entry. Since both NiV and HeV seem to interact with the same two conserved surface residues in ephrinB2 (see below), it suggests that lead compounds identified to inhibit NiV, will also likely inhibit HeV.

CONCORDANCE OF EPHRINB2 EXPRESSION PATTERN WITH KNOWN CELLULAR TROPISM OF NIPAH VIRUS

The expression pattern of ephrinB2 is remarkably concordant with the known cellular tropism of NiV, further underscoring that ephrinB2 is a functional

receptor for NiV *in vivo*. Much of our knowledge regarding ephrinB2 expression *in situ* comes from LacZ reporter mice where the β-galactosidase gene was knocked into the ephrinB2 locus on one chromosome [46–49] (ephrinB2-null mice are embryonic lethal). The correlations between ephrinB2 expression and NiV tropism *in vivo* are listed; direct quotes are taken from some of these primary references to show the remarkable degree of concordance without paraphrasing on our part:

1. EphrinB2 is expressed in neurons and endothelial cells, and is required for axonal guidance and angiogenesis.[36,37,40]
   - NiV has a tropism for neurons and endothelial cells; NiV antigen is found in neurons and endothelial cells; and endothelial syncytia are a hallmark of NiV infection.[18,30]
2. EphrinB2 also selectively marks smooth muscle surrounding arteries but not veins.[48,49]
   - "Vascular staining for Nipah antigen is mainly found in endothelium, endothelial syncytia and smooth muscle of tunica media of small arteries." (FIG. 6 E, F in ref. 18)
3. EphrinB2 is expressed in placental tissue during normal development in all three trimesters.[50]
   - Evidence of HeV placental transmission to fetus in bats and guinea pigs.[35]
4. EphrinB2 expression in spleen is clearly "limited to the central arteries, arterioles, and capillaries of white pulp, terminating abruptly at the marginal zone between white and red pulp" (where venous sinuses originate).[48]
   - In NiV-infected humans (from autopsy samples): "the spleen showed white pulp depletion and acute necrotizing inflammation in the periarteriolar sheaths; viral antigen staining is also particularly evident in periarteriolar sheaths in the white pulp."[18,30]
5. EphrinB2 expression in the sinusoidal lining of lymph nodes can be found.[46]
   - Syncytia were detected in sinusoidal lining of NiV-infected cats[30] and also in subcapsular sinus of infected humans.[18]

ENVELOPE–RECEPTOR INTERACTIONS AND PATHOGENICITY

Receptor Clustering and Signaling

Both ephrinB2, and its cognate endogenous receptor ephB4, have tyrosine signaling and PDZ-binding motifs in their cytoplasmic domains.[38] "Forward" signaling through ephB4 mediates antiadhesive and repulsive behavior upon contact with ephrinB2-expressing cells, while ephrinB2 "reverse" signaling

mediates propulsive adhesion upon contact with ephB4-expressing cells. If NiV-G acts like ephB4 and binds to ephrinB2, but lacks reverse signaling, perhaps only forward propulsion will ensue. We speculate that this might act to recruit more endothelial cells to areas of NiV replication. Indeed, signaling-deficient ephB4 on tumor cells can promote invasion by ephrinB2-expressing endothelial cells.[51] It will be interesting to reexamine pathological specimens for increased angiogenesis in areas of NiV replication. It is also possible that PDZ domain containing proteins and other signaling proteins known to interact with the cytoplasmic domain of ephrinB2 may also play a role in the productive entry of NiV. For example, it has been proposed that PDZ domain containing proteins may bind the cytoplasmic domain of Eph receptor and ephrinB ligands, and stabilize higher-ordered clustering of these receptor ligand pairs into oligomeric arrays.[52] The density of this clustering may modulate the nature and intensity of the forward and reverse signaling.[52] It is known that both HeV-G and NiV-G are tetrameric,[32,53] like the proposed quaternary structures of ephB receptors. Therefore, the clustering of NiV-G with ephrinB2 during the viral entry process may be an essential component of the entry process, and indeed, any putative signals sent through ephrinB2 upon interaction with NiV-G may play a role in viral pathogenesis, as it may allow the cell to become more permissive for viral replication. Since the critical residues in ephrinB2 involved in interactions with NiV-G are the same as those required for interaction with the ephB2 receptor,[33] it is likely that NiV-G will indeed induce reverse signaling via ephrinB2. This is a phenomenon that requires active investigation. Envelope-mediated receptor signaling is an established phenomenon in HIV entry pathogenesis,[54–59] and has even been proposed to play a role in promoting HIV replication in unactivated CD4+ T cells.[60,61]

EphrinB3 as an Alternate Receptor

The virological literature is replete with examples of how minor changes in envelope can lead to attenuation or increased virulence.[62–67] For viruses that can use alternate receptors, which are generally related members of the same family, qualitatively and quantitatively different receptor usage can often account for the different pathogenic profiles. This phenomenon has received much attention in the HIV field where viruses that use CCR5 are generally less cytopathic, and are found earlier on in infection, while viruses that use CXCR4 are generally more cytopathic, and appear with the onset of clinical AIDS.[68–71]

In NiV and HeV infections, despite the similar endothelial cell tropism seen, comparative pathological studies have noted a greater likelihood of meningitis or encephalitic pathology in NiV compared to HeV infections.[30] In pigs, as opposed to most other susceptible animals, the NiV is highly transmissible, with viral antigen readily detectable in respiratory secretions.[29,30] HeV does

not appear to be as readily transmissible,[30,35] although admittedly the number of HeV cases is very small.

Can differential receptor usage contribute to the variant pathogenic profiles observed between NiV and HeV disease? In the published literature, ephrinB2 is clearly the primary receptor used by NiV and HeV in a variety of permissive cell lines.[31,44] In the case for NiV, ephrinB2 also appears to be the receptor used in primary neurons and microvascular endothelial cells.[31] There are at least three ephrinB members in the mammalian genome. While ephrinB1 appears not to support NiV-G binding under the *in vitro* conditions examined,[31,33] ephrinB3 appears to be an alternate receptor that can fully support NiV entry, albeit less efficiently than ephrinB2.[33]

Our first hint that an alternate receptor for NiV may exist was our observation that although ephB4, which binds *only* ephrinB2 *in vivo*, was able to block NiV entry into microvascular endothelial cells,[31] it did not appear to block NiV entry into a subset of embryonic rat neurons (unpublished data). Thus, it is likely that while ephrinB2 is the major receptor for NiV entry into microvascular endothelial cells, NiV can also use an alternate receptor for entry into a particular subset of neurons.

We then formally identified ephrinB3 as a *bonafide* receptor for NiV entry using both Nipah envelope pseudotyped VSV reporter viruses, as well as live Nipah viruses.[33] Using biochemical and biophysical measurements, we also show that NiV-G interacts directly with ephrinB3, but with approximately 10-fold lower affinity than ephrinB2. However, due to the extraordinarily high affinity of NiV-G for ephrinB2 (Kd$\sim$0.06 nM), the affinity of ephrinB3 was still in the subnanomolar range (Kd$\sim$0.6 nM), which may explain why ephrinB3-mediated entry remains readily detectable.[33] In addition, we also pinpointed two key residues common to ephrinB2 and ephrinB3 that mediate their use as receptors for NiV entry. Significantly, these two residues were also critical for their activity as ligands for the endogenous ephB2 receptor (recall that ephrinB ligands bind to ephB receptors). We suggested that ephrinB3 is a relevant receptor for NiV *in vivo*, as MRI lesions in NiV-infected patients can be found in specific regions of the CNS (such as the corpus callosum and the spinal cord)[72,73] that are known only to express ephrinB3, but not ephrinB2.[74,75] Thus, ephrinB3 may serve as a receptor for NiV entry into certain neuronal subsets.

In addition, ephrinB3 expression has been reported in lymphoid cells (T cells),[76] and could also account for acute lymphoid necrosis seen in early NiV infection.[18] While ephrinB2 knockout mice are embryonic lethals with defects in vascular and neuro morphogenesis,[37,40,47–49] ephrinB3 knockout mice are fertile and phenotypically normal, but exhibit peculiar neurological defects arising from the inability of the corticospinal tracts to cross the midline.[77,78] The lack of overt defects in vascular morphogenesis in the ephrinB3 knockout mice suggests that ephrinB3, unlike ephrinB2, is not required for vascular development. Additional support that ephrinB3 is not expressed in

endothelium comes from our observation that ephB4, which only binds ephrinB2, can completely inhibit NiV entry into microvascular endothelial cells.[31] Thus, if ephrinB3 is used as an alternate receptor by NiV and/or HeV, the efficiency by which NiV or HeV uses ephrinB2 versus ephrinB3 could contribute to their differential pathologies. Indeed, since NiV appears to have an increased encephalogenic potential compared to HeV, we speculate that NiV may use ephrinB3 better than HeV, as certain neuronal subsets express both ephrinB2 and ephrinB3 while microvascular endothelial cells only express ephrinB2. This hypothesis is readily testable.

N-GLYCANS ON NIPAH VIRUS ENVELOPE CAN BE RECOGNIZED BY IMMUNE SYSTEM LECTINS

Recently, we have also published that galectin-1 can potently inhibit NiV envelope glycoprotein-mediated cell–cell fusion.[32] In exploring the glycan structures present on NiV-F/G, we noticed that both F and G have polylactosamine sequences that can be bound by galectin-1, an immune system lectin that has myriad functions and is known to be a "tuner" of the inflammatory immune response.[79,80] Since gal-1 is produced by activated endothelial cells[81] (a primary target for NiV infection) and dendritic cells (unpublished observations), we asked whether gal-1 could also serve as an innate immune defense mechanism, by recognizing the pathogen glycan structures on NiV-F/G. We found that gal-1 can potently inhibit NiVF/G-mediated cell–cell fusion in a manner that was dependent on its native homodimeric form, and also dependent on its glycan-binding properties.[32] Importantly, we also determined that gal-1 specifically binds NiV-F and -G and indeed, pinpointed an N-linked glycosylation site on NiV-F that is preferentially bound by gal-1: mutation of that N-linked glycosylation site renders the mutant NiV-F significantly more resistant to gal-1-mediated inhibition. This gal-1-mediated inhibition appears to be relatively specific as gal-1 also inhibited HeV-mediated fusion, but not a variety of other viruses tested.[32] Indeed, gal-1 has been recently shown to *enhance* HIV-1 infection.[82] Thus, gal-1 does not appear to have a ubiquitous antiviral effect, unlike cyanovirin, another lectin that has been shown to inhibit a variety of viruses via steric hindrance mechanisms as a result of binding high-mannose glycans present on their envelope glycoproteins.[83,84] The exact mechanism by which gal-1 inhibits NiV fusion remains to be determined, but since gal-1 can aberrantly oligomerize both NiV-F and G,[32] it is not unreasonable to propose that these aberrant oligomers compromise the ability of the F and G complex to undergo the conformational changes required for membrane fusion.

The concentrations of gal-1 used to inhibit NiV fusion can arguably be found in extracellular matrix.[85,86] If this is so, the question remains as to why the natural amount of gal-1 is not more protective against NiV infection. It is

difficult to answer this teleological question, but perhaps one could also argue that NiV infection would be even more lethal in the absence of endogenous gal-1. In addition, natural polymorphisms in the gal-1 gene, some of which can be predicted to affect the glycan-binding properties of gal-1, may contribute to the variant mortality rates found between the various NiV outbreaks. However, the frequency of these polymorphisms in the affected populations is not known. In addition, it is also not known what the variance of gal-1 levels is in the general population. Perhaps a threshold level of gal-1 is required for its putative protective effect, and these levels are not present in individuals who succumb to the more severe effects of the Nipah viral infection. Indeed, these questions underscore the need to further understand the potential role that innate immune defense mechanisms may play against emerging pathogens.

CONCLUSION

Nipah virus is an emerging pathogen that is likely a zoonosis that occurred as a result of habitat destruction of its primary host, the fruit bat (*Pteropus hypomelanus* and related species).[87] The rapid population growth in South East Asia and the encroachment of human activity into previously undisturbed areas in the region increases the likelihood that more emerging viruses will be discovered. If we are to better control and prevent any future outbreaks, the biological investigation of any new outbreaks must be coupled with an ecological study of the circumstances and parameters that led to these new zoonoses. Thus, the laudable studies by Daszak, Epstein, and colleagues of the Henipavirus Ecology Research Group deserve much attention and support.[88–90]

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